Activate: Randomized Clinical Trial of BCG Vaccination against Infection in the Elderly

Graphical Abstract
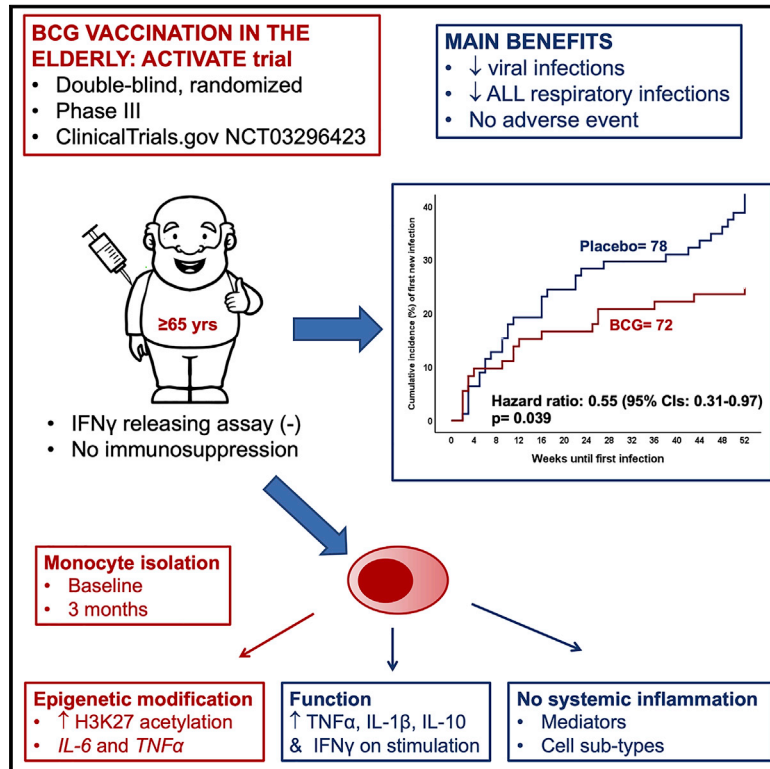
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In Brief

Interim analysis of the phase III ACTIVATE trial to evaluate protection against infection in elderly patients reveals that BCG vaccination is safe, increases the time to first infection, and shows protection against viral respiratory infections.

Highlights

- ACTIVATE is a prospective randomized trial of BCG vaccination in the elderly

- BCG increased the time to first infection and decreased the incidence of new infection

- Strongest protection was found against viral respiratory tract infections

- Epigenetic reprogramming and increased cytokine production was found in monocytes

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Article

Activate: Randomized Clinical Trial of BCG Vaccination against Infection in the Elderly

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SUMMARY

BCG vaccination in children protects against heterologous infections and improves survival independently of tuberculosis prevention. The phase III ACTIVATE trial assessed whether BCG has similar effects in the elderly. In this double-blind, randomized trial, elderly patients (n = 198) received BCG or placebo vaccine at hospital discharge and were followed for 12 months for new infections. At interim analysis, BCG vaccination significantly increased the time to first infection (median 16 weeks compared to 11 weeks after placebo). The incidence of new infections was 42.3% (95% CIs 31.9%–53.4%) after placebo vaccination and 25.0% (95% CIs 16.4%–36.1%) after BCG vaccination; most of the protection was against respiratory tract infections of probable viral origin (hazard ratio 0.21, p = 0.013). No difference in the frequency of adverse effects was found. Data show that BCG vaccination is safe and can protect the elderly against infections. Larger studies are needed to assess protection against respiratory infections, including COVID-19 (ClinicalTrials.gov NCT03296423).

INTRODUCTION

Infection by the novel SARS-CoV-2 virus (also termed COVID-19) has a severe impact on both the health of the populations around the globe, and on the world economy. Many countries are in lockdown, with a third of the world population in some form of movement restrictions, which brings serious financial and societal consequences. The urgent need for the reversal of this situation can be met only through the generation of an immune defense shield to protect the populations from SARS-CoV-2 infection. Many efforts for the development of a vaccine are under way, but it is likely that at least 12–24 months will be needed until an effective vaccine could be available.

Interestingly, however, trained immunity induced by some already available vaccines such as Bacille Calmette-Guérin (BCG), oral polio vaccine (OPV), or the measles vaccine have been suggested to be used as a potential protective approach against COVID-19 to bridge the period until a specific vaccine is developed (Netea et al., 2020). Trained immunity is the process of epigenetic, transcriptional, and functional reprogramming of innate immune cells (such as myeloid cells or natural killer [NK] cells), leading to an increase in the cytokine production capacity

and their antimicrobial function (Kleinnijenhuis et al., 2012; Netea et al., 2016). In models of experimental human infections such as yellow fever vaccine virus (Arts et al., 2018) or human experimental malaria (Walk et al., 2019), BCG vaccination was able to induce a non-specific protection.

These experimental data are accompanied by epidemiological studies in children and adults showing non-specific protection against infections and mortality by BCG vaccination. BCG vaccination reduced the incidence of respiratory syncytial virus infection in children in Africa (Stensballe et al., 2005) and protected the elderly against respiratory tract infections in Indonesia (Wardhana et al., 2011) and Japan (Ohrui et al., 2005). Finally, the concept was also successfully tested in healthy volunteers that were vaccinated with placebo or BCG vaccine and 14 days later received a tri-valent influenza A vaccine. Volunteers previous vaccinated by BCG developed significantly greater titers against hemagglutinin A of the influenza A virus, whereas their circulating monocytes were more potent for the production of interferon-gamma (Leentjens et al., 2015).

ACTIVATE (a randomized clinical trial for enhanced trained immune responses through BCG vaccination to prevent infections of the elderly) is a randomized trial in which hospitalized elderly
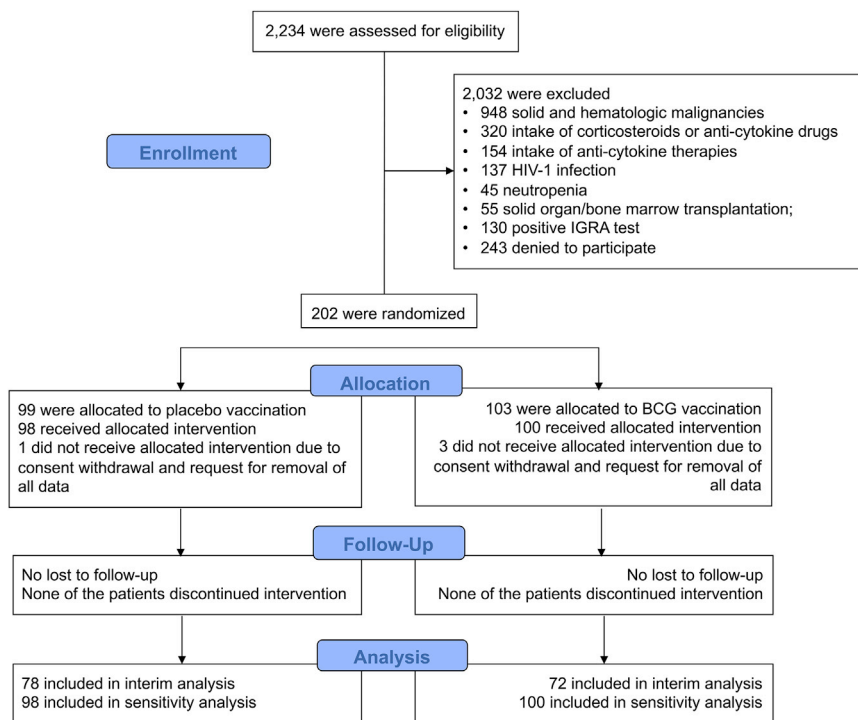
Figure 1. Study Flow Chart
BCG, Bacillus Calmette-Guérin; HIV, human immunodeficiency virus; IGRA, interferon-gamma releasing assay

cebo administration. The incidence of a new infection during the 12-month period of follow-up after vaccination was also significantly decreased; the statistically significant hazard ratio (HR) of 0.55 corresponds to 45% reduction in the risk of a new infection in the BCG group compared to the placebo group (Figure 2A). The incidence of new infection was 42.3% (95% confidence intervals [CIs] 31.9%–53.4%) in the placebo group and 25.0% (95% CIs 16.4%–36.1%) in the BCG group. The difference in the incidence according to the type of infection showed most of the benefit on the prevention of respiratory infections of probable viral origin (Figure 2B); the HR in this case was 0.21 (95% CI 0.06–0.72) corresponding to 79% decrease in the risk for the BCG group in comparison to the placebo group. An analytical presentation of the efficacy of BCG vaccination for all primary and secondary study outcomes is shown in Table 2.

Sensitivity analysis was done for the total of 198 patients taking into consideration that the time of 12-month follow-up has not been completed for 48 patients (Figure 2C; Table S2). This sensitivity analysis confirmed the results of the primary outcome presented in Figure 2A. The confirmation of the primary endpoint of the interim analysis by the sensitivity analysis establishes the absence of any violations on the time-to-event analyses since individuals that are censored have the same probability of experiencing a subsequent event as individuals that remain in the study. The proportionality of the hazards over the total time period of follow-up was validated by plotting the Schoenfeld residuals (Figure S1).

Stepwise Cox regression analysis showed that BCG vaccination was an independent protective factor from the incidence of new infection until month 12 (HR 0.56; 95% CI, 0.32–0.99; p = 0.048) (Table 3).

A major benefit from BCG vaccination was observed in the main secondary endpoint patient-infections per year. This was 57.7 per 100 patients in the placebo group and 33.3 per 100 patients in the BCG group (p = 0.003) (Table 2). No difference in the other secondary endpoints was found between the two groups (Table 2; Figures S2 and S3).

patients were vaccinated on the day of hospital discharge with single doses of placebo or BCG. Patients were under follow-up for 12 months, with the last visit of the last patient scheduled for August 2020. However, the pressure rising from the need of protection of the elderly who are considered susceptible to infection by SARS-CoV-2 (Guan et al., 2020; Huang et al., 2020) led to an interim analysis of the results of the study. Results of this interim analysis clearly showed protection of the elderly from new infections with major effect on the prevention of respiratory infections.

RESULTS

Baseline Characteristics Are Comparable between the Two Arms of Vaccination

From September 2017 through August 2019, 202 patients were enrolled and randomized to double-blind vaccination with placebo or BCG; four patients withdrew consent and requested removal of all data, leaving a final intention-to-treat analysis cohort of 198 patients. No patient was reported as lost to follow-up (Figure 1). Interim analysis included 78 patients allocated to placebo vaccination and 72 patients allocated to BCG vaccination. Baseline characteristics were similar between the two arms (Table 1; Table S1).

BCG Vaccination Decreases the Incidence of New Infections with a Major Impact on the Incidence of Respiratory Tract Infections

Regarding the primary endpoint of the study, BCG vaccination significantly increased the time to first infection: median 16 weeks after BCG vaccine compared to 11 weeks after pla-

The Benefit from BCG Vaccination Is Associated with Modulation of the Pattern of Cytokine Production

In a sub-group of 57 participants (31 placebo and 26 BCG vaccinated), we assessed production of innate immune responses at 2 time points (before and 3 months after vaccination) in

Table 1. Baseline Characteristics of Enrolled Patients

Characteristic	Placebo (N = 78)	BCG (N = 72)	p value
Age, years, mean (SD)	79.6 (7.8)	79.9 (7.6)	0.802
Male gender, no. (%)	35 (44.9)	32 (44.4)	1.000
Charlson's Comorbidity Index, mean (SD)	5.5 (1.9)	5.5 (2.2)	0.909
APACHE II score on study enrolment, mean (SD)	7.9 (3.0)	8.1 (2.9)	0.701
SOFA score on study enrolment, mean (SD)	1.2 (1.4)	1.0 (1.1)	0.586
Comorbidities, no. (%)			
Diabetes mellitus, no. (%)	29 (37.2)	23 (31.9)	0.607
Without organ damage, no. (%)	23 (29.5)	15 (20.9)	0.262
With organ damage, no. (%)	6 (7.7)	8 (11.1)	0.578
Chronic heart failure, no. (%)	23 (29.5)	20 (27.8)	0.858
Chronic renal disease, no. (%)	14 (17.9)	12 (16.7)	1.000
Chronic obstructive pulmonary disease, no. (%)	12 (15.4)	11 (15.3)	1.000
Cerebrovascular disease, no. (%)	17 (21.8)	21 (29.2)	0.349
Degenerative disease, no. (%)	8 (10.3)	6 (8.3)	0.783
Myocardial infarction, no. (%)	13 (16.7)	9 (12.5)	0.498
Biliary stones, no. (%)	10 (12.8)	11 (15.3)	0.814
Renal stones, no. (%)	1 (1.3)	1 (1.4)	1.000
Any surgery, no. (%)	30 (38.5)	30 (41.7)	0.740
Dementia, no. (%)	15 (19.2)	20 (27.8)	0.249
Hemiplegia, no. (%)	1 (1.3)	1 (1.4)	1.000
Peptic ulcer disease, no. (%)	3 (3.8)	3 (4.2)	1.000
Peripheral vascular disease, no. (%)	1 (1.3)	0 (0)	1.000
Liver disease, no. (%)	1 (1.3)	1 (1.4)	1.000
Hypertension, no. (%)	56 (71.8)	53 (73.6)	0.856
Atrial fibrillation, no. (%)	30 (38.5)	22 (30.6)	0.391

APACHE, acute physiology and chronic health evaluation; SD, standard deviation; SOFA, sequential organ failure assessment.

peripheral blood mononuclear cells (PBMCs). Heterologous production of tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and IL-10 (trained immunity induction) (Figures 3A–3E) but not of IL-6 (data not shown) by PBMCs after stimulation with non-mycobacterial ligands was amplified among BCG-vaccinated individuals compared to placebo-vaccinated individuals. A trend toward amplified interferon-gamma (IFN-γ) (heterologous T cell responses) responses was also found (Figure 3F). Unfortunately, the number of BCG-vaccinated individuals in which cytokine data are available is too small to permit the prediction of trained immunity responses as correlates of protection.

Various studies have shown that the increased cytokine responses upon BCG vaccination are the result of epigenetic reprogramming of monocytes (Arts et al., 2018; Kleinnijenhuis et al., 2012). In order to examine potential differences in the epigenetic profile between BCG-vaccinated individuals and controls, we determined at pro-inflammatory genes the level of histone H3 acetylation at lysine 27 (H3K27ac), a mark of active pro-

moters and enhancers. In line with previous findings, we observed increased levels of H3K27ac at the regions of *IL-6* and *TNF-α* in BCG-vaccinated individuals as compared to individuals that received placebo, suggestive of epigenetic reprogramming upon BCG vaccination (Figure 3G).

To further validate the solidity of the observation that BCG induces trained immunity responses in the elderly, we assessed immune responses before BCG vaccination, 2 weeks and 3 months after vaccination in 14 healthy volunteers aged 55 years or older that took part in an independent BCG-vaccination study (300BCG cohort, www.humanfunctionalgenomics.org). All individuals in this cohort were vaccinated with the same BCG strain used in the ACTIVATE trial and PBMCs were isolated and stimulated *ex vivo* with *Staphylococcus aureus*, lipopolysaccharide (LPS), or *Mycobacterium tuberculosis*, before and after vaccination to assess the magnitude of the immune memory responses. We observed a significant increase in IFN-γ upon stimulation with *M. tuberculosis* after BCG vaccination (Figure 3H), indicative of induction of adaptive immune memory response. In addition, cytokine production also significantly increased in the elderly when cells were exposed to non-mycobacterial stimuli such as *S. aureus* and LPS (Figure 3I; Figure S4A), indicative of induction of trained immunity. Furthermore, we observed long-term changes in neutrophil phenotype 3 months upon BCG vaccination as compared to baseline (Figure 3J). Together, these findings indicate sustained trained immunity responses in the elderly and support our previous observation of non-specific beneficial effects against unrelated infections in the elderly upon BCG vaccination. In addition, we employed a targeted proteome platform to measure 92 inflammatory markers before and after BCG vaccination, which revealed no significant changes in the concentrations of circulating inflammatory proteins, including IL-6 and IL-18 after BCG (Figure S4B; Table S3). Similarly, no significant changes in monocyte, granulocyte, or lymphocyte count were observed upon vaccination (Figures S4C–S4E). This demonstrates that, while BCG vaccination induces trained immunity and cell responsiveness, it is not followed by excessive systemic inflammation.

Adverse Events

A trend for lower serious adverse events was recorded in the BCG vaccination group than in the placebo group (Table 4). Moreover, the incidence of non-serious adverse events did not differ between the two groups. None of the adverse events were related to the study intervention. None of the patients developed tuberculosis.

DISCUSSION

The ACTIVATE study was conducted from 2017 with the aim to assess the potential of BCG vaccination to protect the elderly with an increased risk for infection against new infectious episodes. As a target population we have chosen to investigate elderly patients returning home from a hospital admission, as it is known that this population is at a high risk to develop infections (Bender, 2003). This approach using BCG vaccination is justified due to the increasing number of experimental and epidemiological studies suggesting that BCG can protect against respiratory infections in general, and viral infections, in particular (Moorlag et al.,
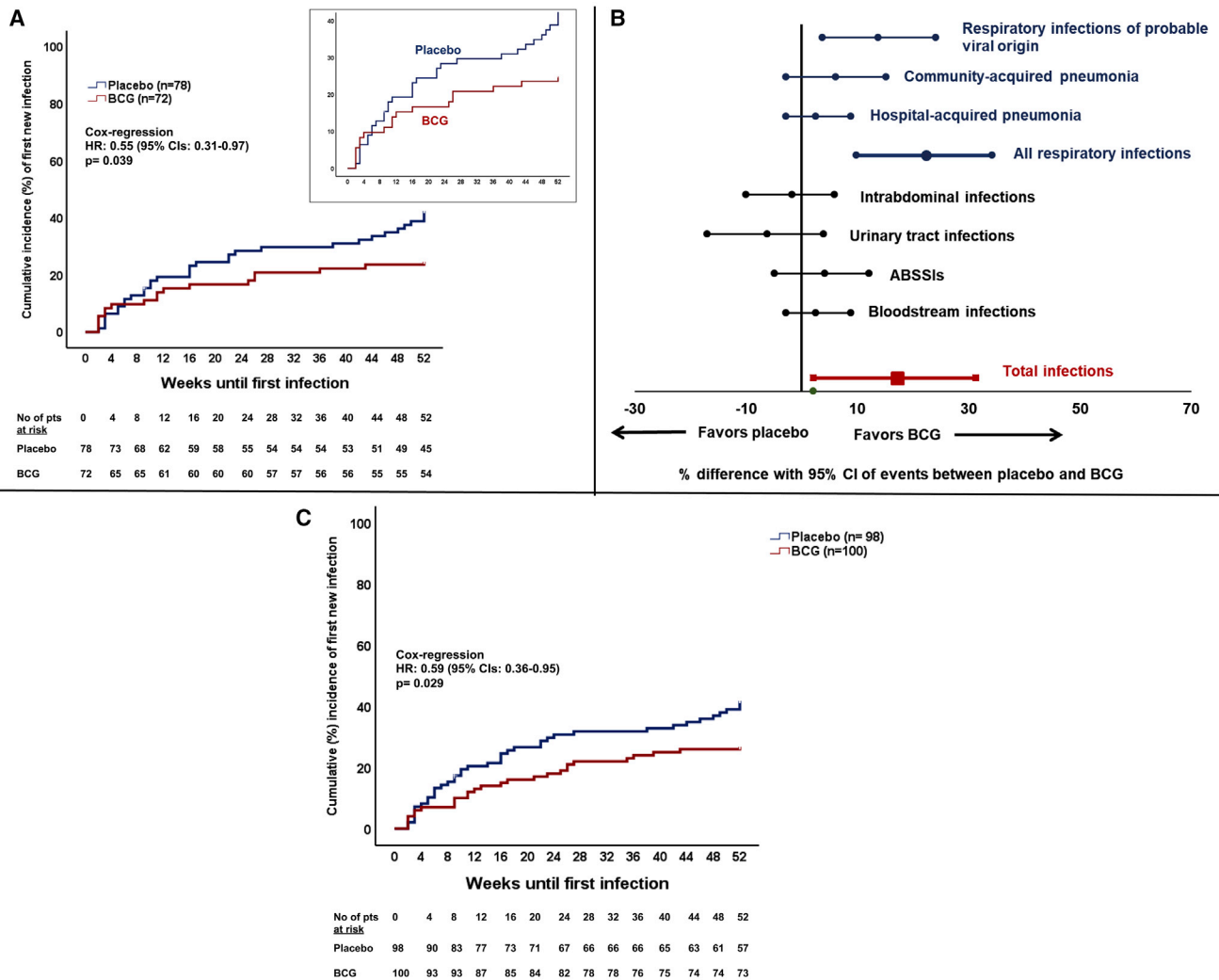
Figure 2. Primary Outcome of the ACTIVATE Trial

(A) Comparative time to first infection in the two groups of vaccination included in the interim analysis (placebo = 78 patients; BCG = 72 patients)

Infections counting against the primary endpoint were respiratory infection of probably viral origin necessitating medical attention, community-acquired pneumonia, hospital-acquired pneumonia, intraabdominal infections, urinary tract infections, bloodstream infections, and acute bacterial skin and skin structure infections (ABSSSIs). The inset shows the same data on an enlarged y axis. The hazard ratio (HR) and the 95% confidence intervals (CIs) of the Cox-regression analysis are shown along with the respective p value of comparison

(B) Percentage differences and 95% CIs of the incidence of each type of infection between the two groups of vaccination included in the interim analysis (placebo = 78 patients; BCG = 72 patients)

(C) Sensitivity analysis: time to first infection after placebo or BCG vaccination among all study participants (placebo = 98 patients; BCG = 100 patients). This analysis is done taking into consideration that the time of 12-month follow-up has not been completed for 48 patients. The HR and the 95% CIs of the Cox-regression analysis are shown along with the respective p value of comparison

2019). Indeed, the data shown here demonstrate that BCG vaccination led to a lower number of infections of all causes, and especially respiratory tract infections, arguing for a protective effect.

Epidemiological data suggest beneficial effects of BCG on all-cause mortality in children in countries with high infectious pressure. This protection has been attributed to lower incidence of neonatal sepsis and respiratory tract infections (Garly et al., 2003), which in children are often viral as etiological cause. This assumption is also supported by the data indicating protective effects of BCG vaccination against RSV infection

(Stensballe et al., 2005). The protection in children was also complemented more recently by studies showing protective effects of BCG vaccination against respiratory tract infections in adolescents (Nemes et al., 2018) and in elderly individuals (Wardhana et al., 2011). In line with this, the incidence of infection in the ACTIVATE trial was significantly lower in the elderly individuals vaccinated with BCG, compared to the non-vaccinated participants. Moreover, this protection was mainly due to respiratory tract infections of probable viral origin, with HR 0.21 in the BCG vaccinated group, which is in line with the

Table 2. Primary and Secondary Study Outcomes

Study Outcome	Placebo (N = 78)	BCG (N = 72)	Hazard Ratio (95% CI)	p value
Incidence at least one new infection until month 12, no. (%)[a]	33 (42.3)	18 (25.0)	0.55 (0.31–0.97)	0.039
Respiratory infections of probable viral origin necessitating medical treatment, no. (%)	14 (17.9)	3 (4.2)	0.21 (0.06–0.72)	0.013
Community-acquired pneumonia, no. (%)	8 (10.3)	3 (4.2)	0.38 (0.10–1.43)	0.153
Hospital-acquired pneumonia, no. (%)	2 (2.6)	0 (0)	–	0.479
All respiratory infections, no. (%)	24 (30.1)	6 (8.3)	0.25 (0.10–0.60)	0.002
Intrabdominal infections, no. (%)	3 (3.8)	4 (5.6)	1.39 (0.31–6.21)	0.667
Urinary tract infections, no. (%)	6 (7.7)	8 (11.1)	1.38 (0.48–3.97)	0.553
Acute bacterial skin and skin structure infections, no. (%)	6 (7.7)	3 (4.2)	0.51 (0.13–2.02)	0.335
Bloodstream infection, no. (%)	2 (2.6)	0 (0)	–	0.497
Incidence of second infection until month 12, no. (%)	9 (11.5)	5 (6.9)	0.59 (0.20–1.77)	0.349
Incidence of third infection until month 12, no. (%)	3 (3.8)	1 (1.4)	0.36 (0.04–3.45)	0.375
Rate of hospitalization/patient until month 12, mean (SD)	0.49 (0.72)	0.43 (0.72)	N/A	0.383
Patient-infections per year, per 100 patients, no. (%)	45 (57.7)	24 (33.3)	N/A	0.003
Courses of antibiotics until month 12, mean (SD)	0.69 (1.66)	0.60 (1.25)	N/A	0.665
One-year mortality, no. (%)	14 (17.9)	10 (13.9)	0.68 (0.30–1.54)	0.356

CI, confidence interval; N/A, not applicable; SD, standard deviation.
[a]Some patients had more than one infection.

70%–80% reduction in respiratory tract infections in studies done in Indonesia and Japan (Ohrui et al., 2005; Wardhana et al., 2011).

An important aspect that should be mentioned is that this interim analysis was performed earlier than the final visit of the planned study, resulting in 78 patients in the placebo group and 72 patients in the BCG group being able to complete the 12-month follow-up. The reason for this interim analysis that has been approved by National Ethics Committee and by the National Organization for Medicines of Greece was the emergence of the COVID-19 pandemic and the initiation of several major studies on the effect of BCG on the infection with SARS-CoV-2. In addition to the effectiveness aspect, another important question that needed to be urgently

answered was that of the safety of BCG vaccination is the setting of COVID-19. An exaggerated inflammatory reaction has been described to contribute to severity and mortality in some patients with COVID-19 (Huang et al., 2020) raising concerns in the community that BCG vaccination may have deleterious effects due to the enhancement of innate immune responses. Indeed, circulating concentrations of proinflammatory cytokines are increased in severely ill COVID-19 patients (Huang et al., 2020). On the other hand, it can be also argued that vaccination with BCG leading to activation of antiviral mechanisms will lead to decreased viral loads and thus lower systemic inflammation, with milder disease and quicker recovery. This model is supported by our earlier studies showing that BCG vaccination decreased viral loads and systemic inflammation after yellow fever vaccine administration (Arts et al., 2018). It is reassuring to observe that this hypothesized protective effect of BCG vaccination is supported by the clinical data in ACTIVATE trial where most of benefit was observed for the incidence of respiratory infection of probable viral origin. Importantly, we observed no increase in the concentrations of pro-inflammatory proteins in the circulation of BCG-vaccinated individuals as compared to concentrations before vaccination, demonstrating that steady-state levels of inflammation are not increased by BCG.

The mechanism of protection induced by BCG vaccination could be either through heterologous T cell responses (Welsh et al., 2010) or though induction of trained immunity (Kleinnijenhuis et al., 2012). Our data showing enhanced cytokine responses to non-mycobacterial stimuli and epigenetic reprogramming of monocytes in BCG vaccinated individuals point toward the induction of trained immunity, although it is likely that a combination between innate and heterologous T cell immunity is responsible for the entire clinical effect.

The main limitations of the trial are (1) the relatively small sample size of our cohort that will need additional validation in a larger study, (B) the lack of repeat IGRA after vaccination, (C) the absence of serological information on the incidence of various respiratory infections, and (4) the lack of information on BCG vaccination at birth. It needs, however, to be mentioned that despite the small sample size significant differences were found.

The number of individuals participating in the trial is too low to permit us drawing any conclusions regarding the effect of BCG vaccination on coronaviruses in general, or COVID-19, in particular. For that, either much longer follow-up or much larger studies are necessary. Indeed, several clinical trials have been started or are under preparations to test this hypothesis. The majority of these trials are studying the protective effect of BCG in healthcare workers, and a synopsis of these trials is provided in Table S4. Serological assessment of the prevalence of antibodies against respiratory coronaviruses is also warranted in future studies.

In conclusion, in the present study we demonstrate that BCG vaccination is safe and decreases the number of infections in an elderly population at risk. While these data need to be interpreted with caution, they support the hypothesis that BCG-induced trained immunity protects against new infections, mostly against respiratory tract infections. Although results argue that BCG vaccination could be used to bridge the period until a specific vaccine for SARS-CoV-2 is developed and produced, larger randomized clinical trials to study the impact of BCG vaccination

Table 3. Univariate and Multivariate Analysis of the Effects of Covariates on the Incidence of at Least One Infection until Month 12

Covariates	No. Infection (n = 99)	At Least One New Infection (n = 51)	Univariate Analysis			Multivariate Analysis		
			HR	95% CI	p value	HR	95% CI	p value
BCG vaccination, no. (%)[a]	54 (54.4)	18 (35.3)	0.55	0.31–0.97	0.035	0.56	0.32–0.99	0.048
Male gender, no. (%)[b]	40 (40.4)	27 (52.9)	0.66	0.38–1.15	0.145			
CCI > 4, no. (%)[c]	61 (61.6)	40 (78.4)	2.11	1.08–4.12	0.028			
Type 2 diabetes mellitus, no. (%)[b]	31 (31.6)	21 (41.2)	1.34	0.76–2.33	0.310			
Chronic heart failure, no. (%)[b]	26 (26.5)	17 (33.3)	1.35	0.75–2.42	0.313			
Chronic renal disease, no. (%)[a]	13 (13.3)	13 (25.2)	1.96	1.05–3.69	0.036	1.95	1.04–3.66	0.038
COPD, no. (%)[a]	10 (10.2)	13 (25.5)	2.14	1.14–4.02	0.018	2.12	1.13–3.99	0.019
Cerebrovascular disease, no. (%)[b]	27 (27.6)	11 (21.6)	0.77	0.40–0.51	0.450			
Degenerative disease, no. (%)[b]	7 (7.1)	7 (13.7)	1.91	0.86–4.25	0.111			
Myocardial infarction, no. (%)[c]	10 (10.2)	12 (23.5)	2.05	1.07–3.92	0.030			
Biliary stones, no. (%)[b]	15 (15.3)	6 (11.8)	0.81	0.34–1.89	0.620			
Any surgery, no. (%)[b]	40 (40.8)	20 (39.2)	0.92	0.53–1.62	0.783			
Dementia, no. (%)[b]	22 (22.4)	13 (25.5)	1.26	0.67–2.37	0.472			
Peptic ulcer disease, no. (%)[b]	4 (4.1)	2 (3.9)	0.88	0.22–3.63	0.863			
Hypertension, no. (%)[b]	75 (76.5)	34 (66.7)	0.69	0.39–1.24	0.215			
Atrial fibrillation, no. (%)[c]	29 (29.6)	23 (45.1)	1.72	0.99–2.99	0.053			

CCI, Charlson's comorbidity index; CI, confidence intervals; COPD, chronic obstructive pulmonary disease; HR, hazard ratio.
[a]Covariates with a significant effect both in the univariate analysis and the multivariate model
[b]Covariates without any significant effect in the univariate analysis and not entered in the multivariate stepwise Cox regression model
[c]Covariates with a significant effect in the univariate analysis but failed to enter significantly in the multivariate stepwise Cox regression model

on morbidity and mortality due to SARS-CoV-2 infection are needed.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead Contact
  - Materials Availability
  - Data and Code Availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - ACTIVATE clinical trial
  - 300BCG study
- METHOD DETAILS
  - ACTIVATE clinical trial
  - 300BCG study
- QUANTIFICATION AND STATISTICAL ANALYSIS
- ADDITIONAL RESOURCES

SUPPLEMENTAL INFORMATION
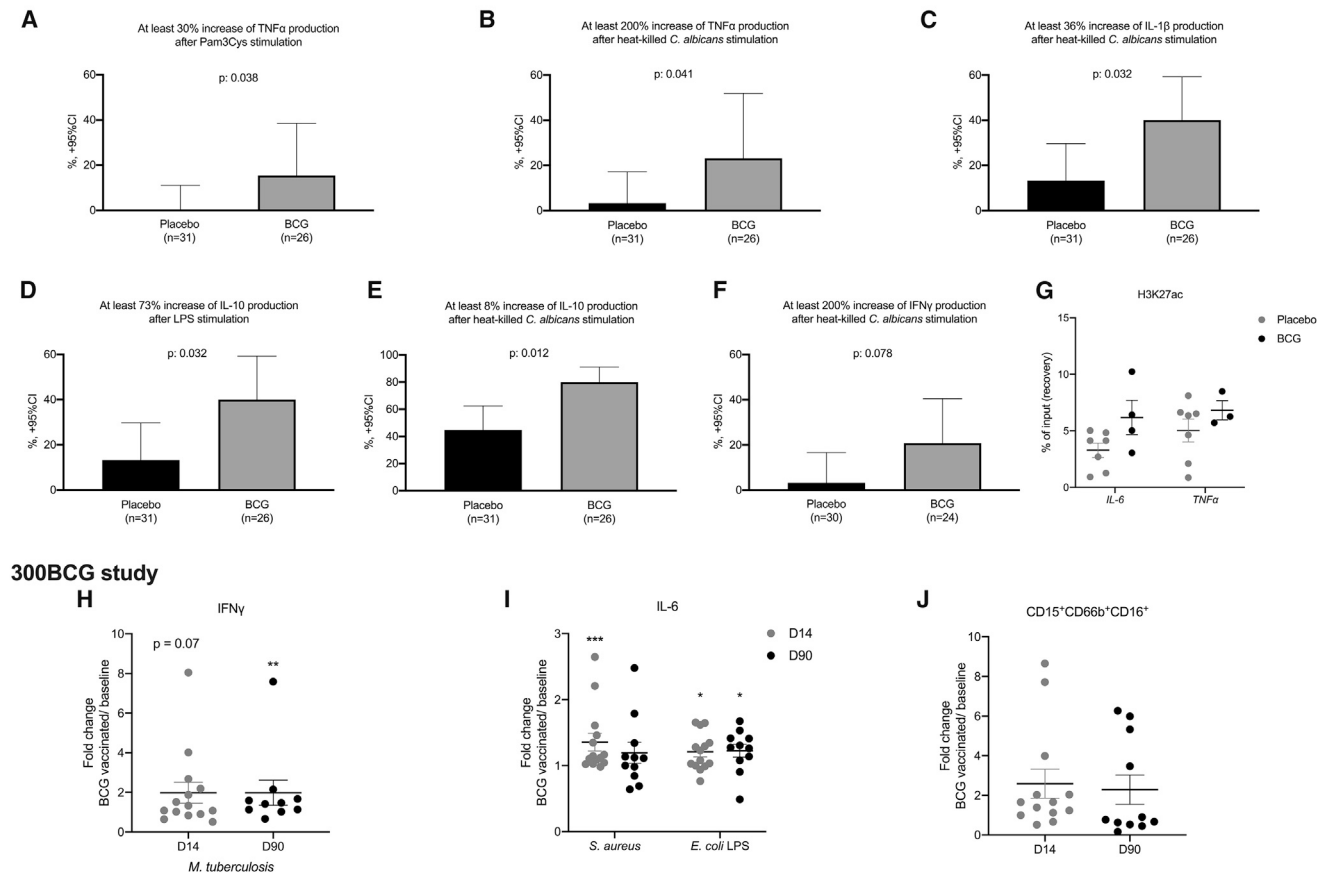
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AUTHOR CONTRIBUTIONS

E.J.G.-B. conceptualized and designed the study protocol, analyzed the data, wrote the manuscript, and gave approval of the final version to be submitted. M.T., N.A., E.K., M.-E.A., A.B., D.-I.D., G.R., and A.P. collected clinical information and blood samples, reviewed the manuscript, and gave approval of the final version to be submitted. A.K. performed study monitoring, reviewed the manuscript, and gave approval of the final version to be submitted. S.M., J.D.-A., T.G., G.D., P.K., A.K., and H.K. performed vaccine accountability and lab experiments, reviewed the manuscript, and gave approval of the final version to be submitted. R.v.C. and M.G.N. conceptualized the study, analyzed the data, drafted the manuscript, and gave approval of the final version to be submitted

DECLARATION OF INTERESTS

ACTIVATE trial

Figure 3. Immunological Effects of BCG Vaccination

Participants in the ACTIVATE trial were vaccinated with placebo or with BCG. (A)–(F) report PBMCs isolated at baseline (month 0) and 3 months after vaccination. PBMCs were stimulated for cytokine production. Blood sampling was not done for all participants after 3 months either because some individuals had died or because they were hospitalized at other study sites or because of denial for blood sampling.

(A) Percentage of patients vaccinated with placebo and BCG with more than 30% increase of the production of TNF-α after stimulation with Pam3Cys.

(B) Percentage of patients vaccinated by placebo and BCG with more than 200% increase of the production of tumor necrosis factor-alpha (TNF-α) after stimulation with heat-killed *Candida albicans*.

(C) Percentage of patients vaccinated by placebo and BCG with more than 36% increase of the production of interleukin (IL)-1β after stimulation with heat-killed *C. albicans*.

(D) Percentage of patients vaccinated by placebo and BCG with more than 73% increase of the production of IL-10 after stimulation with lipopolysaccharide (LPS) of *Escherichia coli*.

(E) Percentage of patients vaccinated by placebo and BCG with more than 8% increase of the production of IL-10 after stimulation with heat-killed *C. albicans*.

(F) Percentage of patients vaccinated by placebo and BCG with more than 200% increase of the production of interferon-gamma (IFN-γ) after stimulation with heat-killed *C. albicans*.

The p values of comparisons by the Fisher's exact test are provided in (A)–(F).

(G) Monocytes were analyzed by chromatin immunoprecipitation (ChIP)-qPCR to determine H3K27ac levels at promoter sites of *IL6* and *TNFα* (n = 7 placebo group, n = 4 BCG group).

Participants in the 300BCG study were vaccinated with BCG

(H and I) PBMCs were isolated and stimulated *ex vivo* with LPS, heat-killed *Staphylococcus aureus*, or *Mycobacterium tuberculosis* before vaccination and 14 days (D14) and 90 days (D90) after vaccination. Fold increases (compared to baseline) of IFN-γ and IL-6 are shown (n = 14, *p < 0.05, **p < 0.01, ***p < 0.001 by the Wilcoxon's signed-rank test.

(J) Cell-surface expression molecules in the granulocyte population were analyzed by flow cytometry before vaccination, and 14 days (D14) and 90 days (D90) after vaccination (fold change of median fluorescence intensity as compared to baseline).

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Table 4. List of Severe Adverse Events and Non-severe Adverse Events Reported during the Study Period

Serious Adverse Events (SAEs)	Placebo (N = 78)	BCG (N = 72)	p value
Presence of at least one SAE,[a] no. (%)	30 (38.5)	17 (23.6)	0.055
Death — no. (%)	8 (10.3)	5 (6.9)	0.568
SAEs with hospitalization,[a] no. (%)	20 (25.6)	10 (13.9)	0.101
Reason for hospitalization, no. (%)			
Arrythmia	1 (1.3)	0 (0)	1.000
Stroke	2 (2.6)	1 (1.4)	1.000
Acute kidney injury	0 (0)	1 (1.4)	0.480
Deep vein thrombosis	1 (1.3)	0 (0)	1.000
Epilepsy	1 (1.3)	0 (0)	1.000
Electrolyte disturbance	1 (1.3)	0 (0)	1.000
Pulmonary edema	1 (1.3)	0 (0)	1.000
Anemia	1 (1.3)	0 (0)	1.000
ST-segment elevation at ECG	1 (1.3)	0 (0)	1.000
Elective surgery	2 (2.6)	2 (2.8)	1.000
SAEs without hospitalization, no. (%)			
Stroke, no. (%)	1 (1.3)	0 (0)	1.000
Syncope	0 (0)	1 (1.4)	0.480
Anemia	1 (1.3)	0 (0)	1.000
Non-serious adverse events (AEs)			
At least one non-serious AE,[a] no. (%)	20 (25.6)	26 (36.1)	0.215
Type of non-serious AE, no. (%)			
Varicella-zoster eruption	1 (1.3%)	0 (0)	1.000
Helicobacter pylori infection	3 (3.8)	0 (0)	0.246
Dacryocystitis	0 (0)	1 (1.4)	0.480
Hip fracture	2 (2.6)	0 (0)	0.490
Non-infection associated cough	4 (5.1)	11 (15.3)	0.055
Asymptomatic bacteriuria	2 (2.6)	7 (9.7)	0.088
Breast cancer	1 (1.3)	1 (1.4)	1.000
Renal cancer	0 (0)	1 (1.4)	0.480
Squamous skin carcinoma	0 (0)	1 (1.4)	0.480
Rash at the injection site	0 (0)	2 (2.8)	0.229
Otitis	0 (0)	1 (1.4)	0.480
Dental infection	2 (2.6)	1 (1.4)	1.000

SAEs and deaths due to infections counting against the primary endpoint are not encountered here since per protocol they should not be reported as adverse events.
[a]Some patients had more than one SAE and/or more than one AE.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-H3K27ac	Diagendode	Cat#pab-196-050: RRID: AB_8580
anti-CD16 FITC (clone 3G8)	Beckman Coulter	Cat#B49215: RRID: AB_2848116
anti-CD10 PE (clone HI10A)	BioLegend	Cat#312203: RRID: AB_314914
anti-CD11b PE-Dazzle (clone ICRF44)	BioLegend	Cat#301347: RRID: AB_2564080
anti-CD14 PECy5.5 (clone M5E2)	BioLegend	Cat#301847: RRID: AB_2564058
anti-CD62L PE-Cy7 (clone DREG-56)	BioLegend	Cat#301347: RRID: AB_830800
anti-PD-L1 APC (clone MIH1)	ThermoFisher	Cat#17598342: RRID: AB_10597586
anti-CD66b AF700 (clone G10F5)	BioLegend	Cat#305113: RRID: AB_2566037
anti-CD15 Brilliant Violet 421 (clone W6D3)	BioLegend	Cat#323039: RRID: AB_2566519
anti-CD45 Krome Orange (clone J33	Beckman Coulter	Cat#A96416: RRID: AB_2833027
Bacterial and Virus Strains		
Heat-killed *Mycobacterium tuberculosis*	Gift	H37Rv
Bacille Calmette-Guérin Vaccine	Intervax	Bulgaria strain
Heat-killed *Staphylococcus aureus*	Gift	Clinical isolate
Chemicals, Peptides, and Recombinant Proteins		
RPMI 1640 W/ Stable Glutamine W/ 25 MM HEPES	Biowest	L0496
PBS Dulbecco's Phosphate Buffered Saline w/o Magnesium, w/o Calcium	Biowest	L0615
FBS Superior; standardized Fetal Bovine Serum, EU-approved	Biochrom	S0615
Gentamycin Sulfate BioChemica	PanReac AppliChem	A1492
Penicillin G Potassium Salt BioChemica	PanReac AppliChem	A1837
Lipopolysaccharides from *Escherichia coli* O55:B5	Sigma-Aldrich	L2880
Ficoll-Paque	GE Healthcare	Cat#17-1440-03
Roswell Park Memorial Institute medium (RPMI)	Invitrogen	Cat#22406031
16% Formaldehyde	Fisher Scientific	Cat#28908
Protein A/G Magnetic beads	Diagenode	Cat#C03010021-150
Bovine Serum Albumin (BSA)	Sigma-Aldrich	Cat#A7030
Protease inhibitor cocktail	Sigma-Aldrich	Cat#P8465
Ficoll-Paque	GE Healthcare	Cat#17-1440-03
Critical Commercial Assays		
Human TNFa uncoated ELISA	Invitrogen	88-7346
Human IL-6 ELISA	Invitrogen	88-7066
Human IL-1b uncoated ELISA	Invitrogen	88-7261
Human IL-10 uncoated ELISA	Invitrogen	88-7106
Human IFNγ ELISA	Diaclone	950.000.192
QuantiFERON®-TB Gold (QFT®) Tubes	QIAGEN	590-0301
QuantiFERON®-TB Gold (QFT®) ELISA	QIAGEN	0594-0201
MinElute PCR purification Kit	QIAGEN	28006
Olink Inflammation panel	Olink Proteomics	95302

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
Primers for qPCR, see STAR Methods	This paper	N/A
Software and Algorithms		
GraphPad Prism	Graphpad Software	https://www.graphpad.com
SPSS	IBM	https://www.ibm.com/analytics/spss-statistics-software
Cytobank platform	Beckman Coulter	https://www.beckman.com/flow-cytometry/software/cytobank-premium/premium-v-enterprise

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Evangelos J. Giamarellos-Bourboulis (egiamarel@med.uoa.gr).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

Data of this study are available after communication with the Lead Contact. A material transfer agreement will be needed.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

ACTIVATE clinical trial

ACTIVATE was a prospective, double-blind, randomized and placebo-controlled phase III clinical trial conducted among patients of both genders hospitalized at the 4th Department of Internal Medicine of ATTIKON University General Hospital in Greece. The protocol and its subsequent amendments were approved by the National Ethics Committee and by the National Organization for Medicine of Greece, and by the Ethics Committees of ATTIKON hospital (EudraCT number, 2017-000596-87). The trial is conducted and funded by the Hellenic Institute for the Study of Sepsis. The funders have no role in the design, conduct, analysis and interpretation of data, and decision to publish. The laboratory of Immunology of Infectious Diseases of the 4th Department of Internal Medicine at ATTIKON University General Hospital served as a central laboratory for the study. The Sponsor initiative for an interim analysis was submitted to the National Ethics Committee and to the National Organization for Medicine of Greece on April 14th 2020 informing on the reasons for the analysis and on the procedure to be followed. The analysis was done by an independent committee of three experts who were appointed by the Sponsor through an invitation on April 16th 2020. The database lock was done on April 21st 2020 and the interim analysis was done by the Committee on April 29th 2020. The investigators remained blind to the intervention after the results of the analysis became known.

The study enrolled elderly patients (age ≥ 65 years) of both genders who were discharged from hospital after hospitalization for a medical cause. Exclusion criteria were: a) denial for written informed consent; b) solid organ malignancy or lymphoma diagnosed the last five years; c) treatment with oral or intravenous steroids defined as daily doses of 10mg prednisone or equivalent for longer than the last 3 months; d) severe immunodeficiency including infection by the human immunodeficiency virus (HIV-1), neutropenia (less than 500 neutrophils/mm^3), history of solid organ and bone marrow transplantation, intake of chemotherapy, primary immunodeficiency, severe lymphopenia (less than 400 lymphocytes/mm^3) and treatment with anti-cytokine therapies; and e) positive Interferon-gamma Release Assay (IGRA). All patients or their legal representatives provided written informed consent before enrolment STAR Methods.

On the day of hospital discharge and after careful recording of detailed medical history and laboratory examinations for the inclusion and exclusion criteria, patients remaining eligible underwent IGRA test. Those who had negative IGRA were allowed to be enrolled in the study. Participants were randomized to one intradermal vaccination with 0.1ml of sodium chloride 0.9% or with 0.1ml of BCG (BCG vaccine strain 1331; Intervax). Simple randomization was performed in a 1:1 ratio by a biostatistician and delivered to the investigators as an electronic file for treatment allocation at randomization. Blind administration was secured by two study pharmacists; one who was preparing the vaccine and another who was delivering the preparation to the investigators for vaccination.

The primary outcome was the time interval to the first infection post hospital discharge between the two groups of treatment. This was a composite endpoint involving any of the following infections: respiratory tract infections of probable viral origin necessitating medical attention; bloodstream infection; community-acquired pneumonia; hospital-acquired pneumonia; urinary tract infections; intraabdominal infection; and soft-tissue infection. Respiratory infections of probable viral origin necessitating medical attention were defined as body temperature above 38°C accompanied by at least two of the following: redness and purulent discharge of the throat; intense nasal discharge; cough; enlargement of cervical lymph nodes; enlargement of liver or of spleen found on deep abdominal palpation; and absolute lymphocyte count more than 4,000/mm^3 (Antonopoulou et al., 2012). Bloodstream infection was defined as at least one positive blood culture for a pathogen not related to infection at other site (Calandra et al., 2005). Community-acquired pneumonia was defined as any new or evolving infiltrate on chest X-ray in a patient without any contact with the hospital environment the last 90 days and who was presenting with at least two of the following: new onset or worsening of cough; body temperature above 38°C; dyspnea; purulent expectoration; auscultatory findings compatible with pulmonary consolidation; and procalcitonin $\geq$ 0.25 ng/ml or absolute total white blood cell count $\geq$ 12,000/mm^3 (Christ-Crain et al., 2006). Hospital-acquired pneumonia was defined as any new or evolving infiltrate on chest X-ray in a patient starting at least 48 hours after hospital admission and who was presenting with at least two of the following: new onset or worsening of cough; body temperature above 38°C; dyspnea; purulent expectoration or tracheobronchial secretions; auscultatory findings compatible with pulmonary consolidation; and procalcitonin $\geq$ 0.25 ng/ml or absolute total white blood cell count $\geq$ 12,000/mm^3 (Kalil et al., 2016). The urinary tract infection was defined as the presence of $\geq$ 10 leukocytes/high power field in urine sediment or of positive urine culture with $\geq$ 10^5 colonies/ml in a patient who was presenting with at least two of the following: body temperature above 38°C; dysuria, increased urinary frequency or urgency; flank pain or lumbar pain at palpation; and ultrasound findings compatible with acute pyelonephritis (Pinson et al., 1997). One intraabdominal infection was defined as any radiological documentation of intraabdominal infection by abdominal X-ray or computed tomography of the abdomen accompanied by at least two of the following: body temperature above 38°C; nausea or vomiting; abdominal pain; and jaundice (Calandra et al., 2005). Acute bacterial skin and skin structure infection was defined as the acute onset of infection involving one upper extremity or one lower extremity or the abdominal wall accompanied by at least two of the following: redness, hotness and edema; well-circumscribed rim; ultrasound findings compatible with soft tissue infection; and absolute total white blood cell count $\geq$ 12,000/mm^3 (Russo et al., 2016).

Secondary study outcomes included the rate of hospitalizations until month 12; the time to first sepsis episode; the total number of infections; the time to first hospitalization; the number of antibiotic courses; and one-year mortality. Sepsis was defined by the Sepsis-3 definitions, i.e., $\geq$ 2-point increase of the sequential organ failure assessment (SOFA) score measured on the day of study enrolment (Singer et al., 2016). For the analysis of both the primary and the secondary endpoints only events captured during the first 12 months after vaccination with placebo or BCG were encountered.

Patients' visits were conducted every month for 12 months. During each visit the following data were captured: history of any new infection; any new hospitalization followed by thorough study of hospital discharge notes and contact with treating physicians if necessary; any need for antimicrobial prescription; and patient disposition. Ten ml of whole blood was sampled after venipuncture of one forearm vein under aseptic conditions before vaccination and repeated three months after vaccination with placebo/BCG. Blood was immediately transferred to the central lab for the isolation and culture of PBMCs.

Adverse events (AEs) and Serious Adverse Events (SAEs) were captured from baseline until the last patient's evaluation. An adverse event was defined as any undesirable medical occurrence in a subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. The adverse event could be a sign, a symptom, or an abnormal laboratory finding. AEs meeting any of the following criteria were considered SAEs: death; life-threatening situation; inpatient hospitalization or prolongation of existing hospitalization; persistent or significant disability/incapacity; congenital anomaly/birth defects; important medical events/experiences; and spontaneous and elective abortions experienced by study subject. All others AEs were reported as non-serious. All AEs were graded as: mild (transient and well-tolerated by the patient), moderate (causing discomfort and affecting the usual activities of the patient) and severe (affecting the usual activities to an important degree and causing disability or are life-threatening). The relationship of the AE to the study drug was reported as probably-related, possibly-related; probably not-related and unrelated

300BCG study

To study the immunological effects of BCG vaccination in elderly, immune responses were assessed in 14 healthy volunteers (4 females, 10 males) aged 55 years or older, that took part in the 300BCG study (ethical approval no. NL58553.091.16). Whole blood was drawn by venipuncture of one forearm vein under aseptic condition and collected into BD Vacutainer® spray-coated EDTA tubes. Blood sampling was done before BCG vaccination, two weeks and three months after vaccination. All volunteers received 0.1ml of BCG (BCG vaccine strain 1331; Intervax). Three volunteers did not show up at their three-month appointment, therefore the time point three months after vaccination contains data of 11 volunteers. The study was approved by the Ethical Committee (CMO) of Radboud University Nijmegen, the Netherlands. Inclusion of volunteers and experiments were conducted according to the principles expressed in the Declaration of Helsinki and all volunteers gave written informed consent before any material was taken.

METHOD DETAILS

ACTIVATE clinical trial

For the IGRA test, venous blood was collected in four heparinized tubes, namely: one negative control; two tubes containing *M. tuberculosis*-specific antigens (ESAT-6, CFP-10); and one tube contained a positive control mitogen. Following incubation and centrifugation of the tubes, plasma was removed and the concentration of IFNγ produced in each tube was measured by an enzyme immunosorbent assay (QuantiFERON®-TB Gold, QFT® ELISA, QIAGEN). For the interpretation of the IGRA assay test, concentrations of IFNγ in the negative control were subtracted from the levels of antigen and mitogen stimulation respectively. The test was considered positive if IFNγ after antigen stimulation was higher than the negative control.

Peripheral blood mononuclear cells (PBMCs) were isolated from participants of the ACTIVATE study after gradient centrifugation over Ficoll (Biochrom, Berlin, Germany) for 20 minutes at 1400 g. After three washings in ice-cold PBS pH 7.2, PBMCs were counted in a Neubauer plate with trypan blue exclusion of dead cells. They were then diluted in RPMI 1640 enriched with 2mM of L-glutamine, 500 μg/ml of gentamicin, 100 U/ml of penicillin G, 10 mM of pyruvate, 10% fetal bovine serum (Biochrom) and suspended in wells of a 96-well plate. The final volume per well was 200 μL with a density of 2 x10^6 cells/ml. PBMCs were exposed in duplicate for 48 hours and 5 days at 37°C in 5% CO_2 to different stimuli: 10 ng/ml of *Escherichia coli* O55:B5 lipopolysaccharide (LPS, Sigma, St. Louis, USA); 5 μg/ml of Pam3Cys-SKKK (EMC Microcollections, Tübingen, Germany); and 5x10^5 colony forming units of heat-killed *Candida albicans*. Following incubation, cells were removed and analyzed for flow cytometry. Concentrations of TNFα, IL-1β, IL-6, IL-10 and IFNγ were measured in cell supernatants in duplicate by an enzyme immunosorbent assay (Invitrogen, Carlsbad, California, USA). The lowest detections limits were: for TNFα 40 pg/ml; for IL-1β 20 pg/ml; for IL-6 10 pg/ml; for IL-10 157 pg/ml; and for IFNγ 12.5 pg/ml.

For chromatin immunoprecipitation, purified monocytes were fixed with 1% formaldehyde (Sigma) at concentration of 10^6 cells/ml. Fixed cell preparations were sonicated using a Diagenode Bioruptor UCD-300 for 3 × 10 min (30 s on; 30 s off) and incubated with protease inhibitor cocktail and H3K27ac antibody (Diagenode) and incubated overnight at 4°C with rotation. Protein A/G magnetic beads were washed in dilution buffer with 0.15% SDS and 0.1% BSA, added to the chromatin/antibody mix and rotated for 60 min at 4°C. Following five washes, chromatin was eluted using elution buffer for 20 min. Supernatant was treated with proteinase K for 4 h at 65°C. Finally, samples were purified using QIAGEN; Qiaquick MinElute PCR purification Kit. Primer pairs used for quantitative PCR were as follows: *IL-6* forward: 5′-TGC ACA AAA TTT GGA GGT GA-3′; *IL-6* reverse: 5′-ACC CAA CCT GGA CAA CAG AC-3′; *TNFα* forward: 5′- CTT GGG CCA GTG AGT GAA AG-3′; *TNFα* reverse: 5′-TAG CCA GGA GGG AGA ACA GA.

PBMCs were isolated from the whole blood of participants of the 300BCG study as described above. PBMCs were stimulated at counts of 5 × 10^6/ml in 96-well plates by 5 μg/ml of heat-killed *M. tuberculosis* H37Rv, 5x10^6 cfu/ml heat-killed *S. aureus* or 10 ng/ml of *E. coli* LPS. After 24 hours and 7 days of incubation at 37°C, supernatants were collected and stored at −20°C until analysis. Concentrations of IL-1β and IL-6 were measured in 24-hour supernatants and of IFNγ in 7-day supernatants by the enzyme immunosorbent assay described above.

300BCG study

Using whole blood of participants in the 300BCG study, blood cells were counted using a Coulter Ac-T Diff® cell counter (Beckman Coulter, Brea, USA). Following red blood cell lysis, white blood cells were washed twice with PBS and re-suspended in 300 μL of PBS + 0.2% BSA (Sigma-Aldrich, Zwijndrecht, Netherlands) and stained with the following monoclonal antibodies: anti-CD16 FITC (clone 3G8, Beckman Coulter; Suarlée, Belgium), anti-CD10 PE (clone HI10A, BioLegend, San Diego, CA, USA), anti-CD11b PE-Dazzle (clone ICRF44, BioLegend), anti-CD14 PE-Cy5.5 (clone M5E2, BioLegend), anti-CD62L PE-Cy7 (clone DREG-56, BioLegend), anti-PD-L1 APC (clone MIH1, ThermoFisher, Waltham, MA USA), anti-CD66b AF700 (clone G10F5, BioLegend), anti-CD15 Brilliant Violet 421 (clone W6D3, BioLegend) and anti-CD45 Krome Orange (clone J33, Beckman Coulter). Cells were analyzed on a 10-color Navios flow cytometer (Beckman Coulter). Acquired FACS data were analyzed using the cloud-based Cytobank platform.

Circulating plasma inflammatory markers were assessed in the plasma of participants of the 300 BCG study using the commercially available Olink Proteomics AB (Uppsala Sweden) Inflammation panel (92 inflammatory proteins), using a Proseek© Multiplex proximity extension assay (Assarsson et al., 2014). Of the 92 proteins in the panel, 73 proteins were detected in the plasma samples and included in the analysis. See Table S3 for an overview of all inflammatory markers that were analyzed.

QUANTIFICATION AND STATISTICAL ANALYSIS

The trial sample size was calculated assuming the median time to new infection would be 4 months in the placebo group and 7 months with BCG vaccination. To achieve so with 90% power at the 5% level of significance, 100 patients were allocated to each arm. Under these prerequisites, the study is sufficiently powered to prove that differences in the first time incidence of infection between the placebo and the BCG group of the order of 20% will be statistically significant.

Baseline qualitative data were presented as percentages and CIs and compared by the Fisher's exact test. Baseline quantitative data were presented as mean and standard deviation and compared by the Student's "t-test" for variables that followed normal distribution; they were presented as mean and standard error and compared by the Mann-Whitney U test for variables that did not follow normal distribution.

The primary outcome was the time, in weeks, of the appearance of one first new infection, censored at twelve months after vaccination. Differences between the placebo and BCG vaccination groups were assessed with the HR of the Cox proportional hazards regression model with its 95% CIs. The corresponding p values were also reported. The effects of other confounders, both at the univariate and the multivariate Cox model, were also assessed with the corresponding HR. Only variables found to be significant in the univariate analysis entered in the stepwise multivariate analysis and they were retained in the model only if they had a significant effect after adjusting for the other effects. The proportionality of the hazard function at different levels throughout the follow-up period was assessed with the Schoenfeld residuals method (Xue et. al., 2013). Since this was an interim analysis, a sensitivity analysis was also performed for the total number of participants with the primary aim to show that individuals that are censored have the same probability of experiencing a subsequent event as individuals that remain in the study. The number of infections in each group was expressed as patient-infections per year. The same analysis was done for the secondary endpoints. The frequency of adverse events was compared by the Fisher's exact test.

The interim analysis included only patients with completed 12-month of follow-up. In order to preserve the overall Type I error rate at 5%, an adjustment of the level of significance of the interim and final analyses was done by O'Brien-Fleming strict alpha adjustment. This adjustment provides significance a = 0.0054 at interim and a = 0.0492 at final (DeMets and Lan, 1994). The purpose of using this seemingly unattainable level of significance at interim analysis was to allow the study to conclude, at the same time providing evidence that the required level of significance will be attained at the final stage. Statistical analysis was performed with the IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA) and corroborated with the R statistical package (R Core Team, 2013) (see supplementary Statistical Analysis plan, Data S1 and Methods S1).

Cytokine data were expressed for each group of vaccination as the ratio of the cytokine production at month 3 versus the production at month 0 (before vaccination). Receiver operator characteristic curve analysis was done to discriminate the ratio of each cytokine that can better differentiate the two groups of vaccination. The best cut-off of this ratio was selected by the co-ordinate points of the curve using the Youden index. Patients above and below this cut-off were compared between groups by the Fisher's exact test.

Cytokine and protein data of participants in the 300BCG study were expressed as means ± SE. Data were analyzed using the Wilcoxon's rank sum test.

Analysis was conducted using IBM SPSS Statistics v. 25.0. All p values were two-sided and any p value < 0.05 was considered as statistically significant.
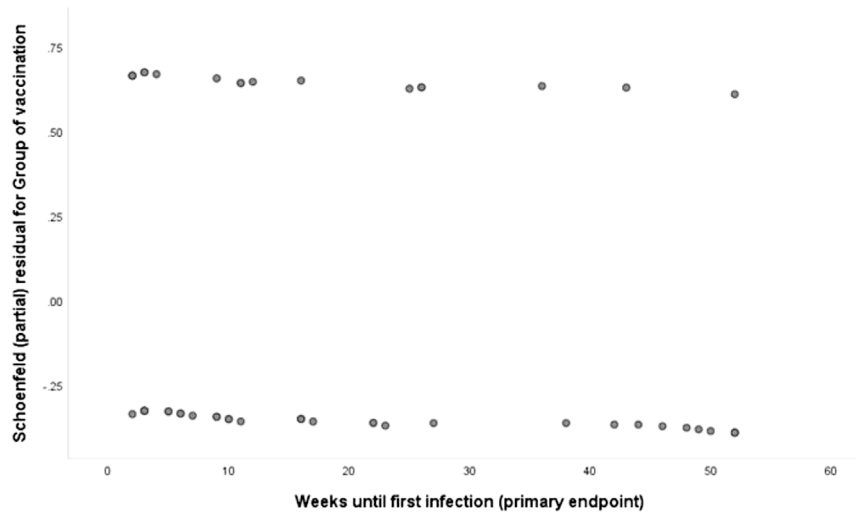
Ongoing interventional clinical trials studying the protective effect of BCG vaccination on Covid-19 were reviewed using the Clinicaltrials.gov registry of trials (see Table S4).

ADDITIONAL RESOURCES

The study is registered at ClinicalTrials.gov NCT03296423

Supplemental Figures

Figure S1. Plot of Schoenfeld Residuals against Time to Event, Related to Figure 2

If the assumption of proportionality of hazards across the two groups is not violated then residuals for the two groups should lie in two parallel straight lines. The above chart confirms this. Therefore the assumption of proportionality of the hazards across the whole time of follow-up is not violated since it remains practically the same.

Cox regression
HR: 0.74 (95% CIs 0.42=1.31)
p: 0.298
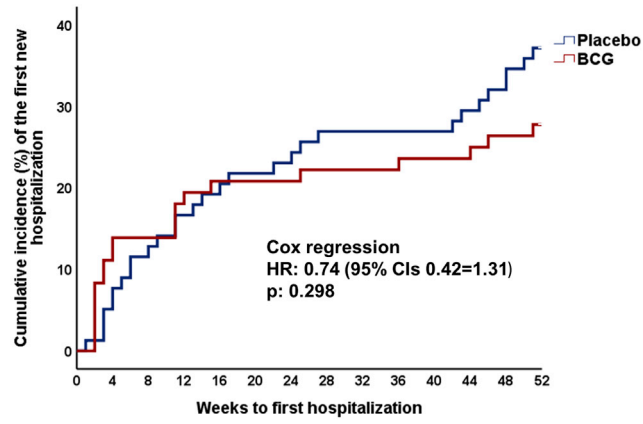
No of pts at risk	0	4	8	12	16	20	24	28	32	36	40	44	48	52
Placebo	78	72	68	65	62	61	59	57	57	57	57	55	50	49
BCG		72	62	62	58	57	57	57	56	56	55	55	54	53	52

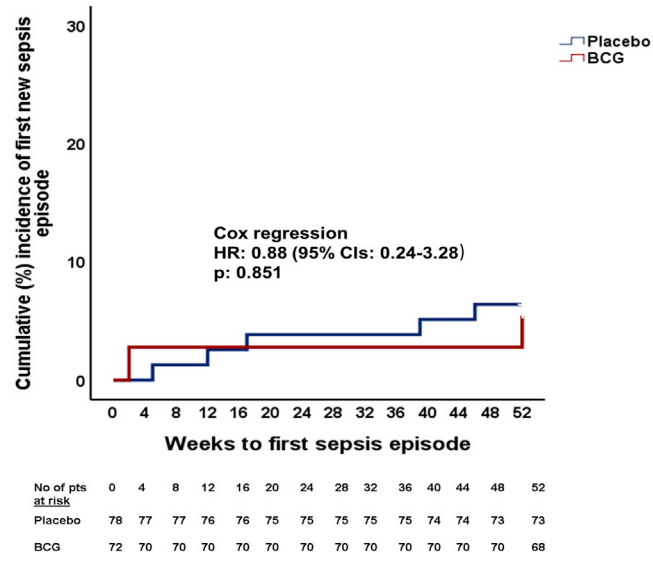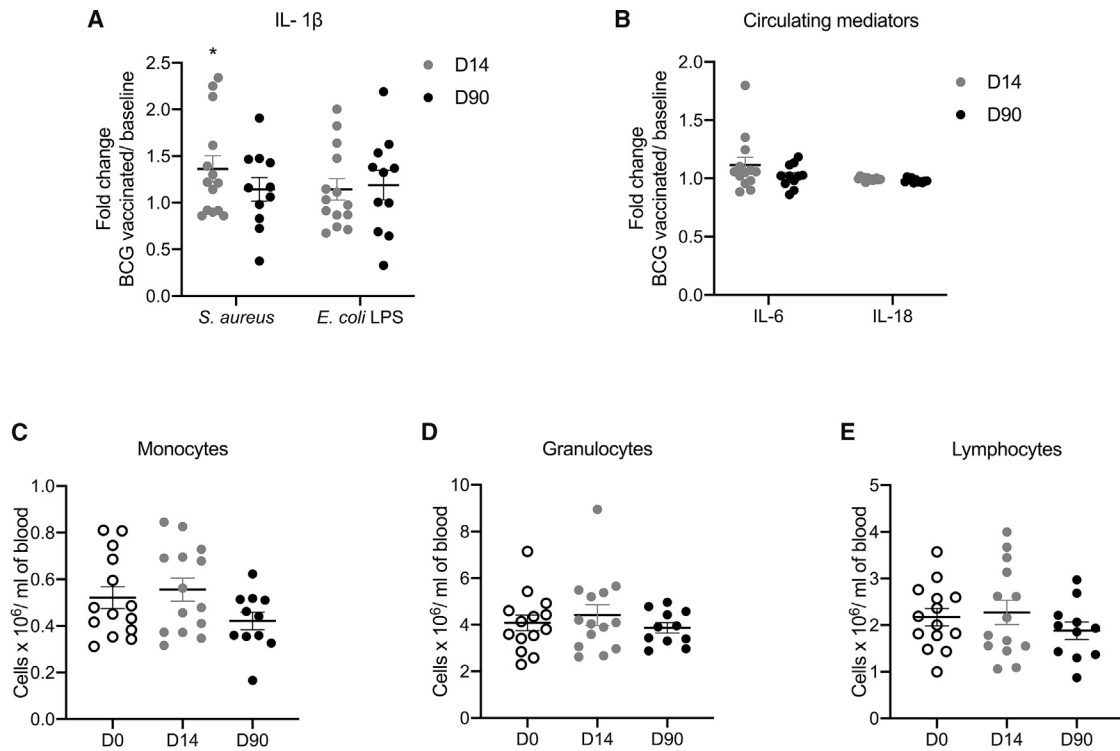**Figure S2. Time to First Hospitalization after Placebo or BCG Vaccination, Related to Table 2**

Figure S3. Time to First Sepsis Episode after Placebo or BCG Vaccination, Related to Table 2

Figure S4. Immunological Effects of BCG Vaccination in the Elderly, Related to Figure 3

(A) Healthy volunteers (≥55 years) from the 300BCG cohort were vaccinated with BCG and peripheral blood mononuclear cells (PBMCs) were isolated and stimulated *ex vivo* with *S. aureus* or lipopolysaccharide (LPS) of *Escherichia coli* before vaccination, 14 days (D14) and 90 days (D90) after vaccination. Fold increases (compared to baseline) of interleukin (IL)-1β are shown (n = 14, *p < 0.05 by the Wilcoxon's ranked sum test). (B) Fold changes of circulating IL-6 and IL-18 upon BCG vaccination as compared to baseline (n = 13). See also Table S3 for a complete list of circulating markers that were measured. (C) Absolute monocyte, granulocyte and lymphocyte counts in whole blood before vaccination, 14 days (D14) and 90 days (D90) after vaccination. Mean values and SE are provided in each panel.